Comparison of Zinc Acetate and Propionate Addition on Gastrointestinal Tract Fermentation and Susceptibility of Laying Hens to *Salmonella enteritidis* During Forced Molt

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ABSTRACT Feed deprivation is the most common method used to induce molting and stimulate multiple egg-laying cycles in laying hens for commercial egg production. Unfortunately, an increased risk of *Salmonella enteritidis* (SE) colonization may result from the use of this method. Methods to stimulate multiple egg-laying cycles without increasing the risk of SE are needed. In each of 3 experiments, hens over 50 wk of age were divided into groups of 12 and placed in individual laying cages. One week before dietary changes, hens were put on an 8L:16D photoperiod that continued for the 9-d experimental period. Hens in all treatments were challenged orally with 10^4 cfu of SE on the fourth day. Treatments were full fed hens (nonmolted, NM), nonfed hens (molted, M), a zinc acetate diet (ZAC), and a zinc propionate diet (ZPR). The zinc diets contained 10,000 mg of zinc per kilogram of diet.

Body weight losses were significantly higher in the M, ZPR, and ZAC treatments than in the NM treatment. Crop lactic acid decreased more in M, ZPR, and ZAC treatments than in NM hens in trial 2. Crop pH was significantly ($P < 0.05$) lower in NM hens than in M, ZAC, and ZPR hens in trial 2. Although cecal individual or total volatile fatty acids (VFA), and lactic acid were not significantly ($P > 0.05$) different between NM hens and M, ZAC and ZPR hens in trial 1, lactic acid was significantly ($P < 0.05$) higher in NM hens than in M, ZAC and ZPR hens (trial 2), and cecal total VFA were lower in M hens than in NM, ZAC and ZPR hens (trial 3). Colonization of SE in the crop and ceca was higher in the M and ZPR hens (trials 1 and 2). Liver, spleen, or ovary invasion by SE was higher in the M and ZPR hens (trials 1 and 2) than in NM hens. At the zinc concentration used in these studies, the zinc dietary regimens may be effective for reducing the risk of SE during induced molt.

(*Key words*: laying hen, molting, *Salmonella enteritidis*, zinc acetate, zinc propionate)

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INTRODUCTION

Salmonellosis is one of the most common foodborne diseases with an estimated 800,000 to 4 million human infections reported each year in the US alone. During the past 10 to 15 yr, the number of cases of gastroenteritis due to *Salmonella enterica* subspecies *enterica* serovar Enteritidis (SE) infections has increased markedly in the US and Europe; by 1995, SE comprised 25% of all foodborne *Salmonella* isolates (Holt et al., 1995). Between 1985 and 1991, 82% of SE infections in the US were associated with table eggs (St. Louis et al., 1988). *S. enteritidis* is invasive in poultry and therefore has the potential to contaminate eggs by transovarian transmission following colonization of the intestinal tract (Thiagarajan et al., 1994).

It has been suggested that the high incidence of SE infection may be linked to the specific stressful management practice of inducing a molt to stimulate multiple egg-laying cycles in hens (Holt, 2003). According to the National Animal Health Monitoring system (NAHM) Layers 1999 report, more than 94% of commercial laying facilities in the western US use induced molting as a means of increasing productivity in flocks. Feed withdrawal is the primary method used to induce molting but has been shown experimentally to increase SE recovery from crops, increase invasion of organs in chickens

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Abbreviation Key: BGA = brilliant green agar; M = molted; NONA = novobiocin-nalidixic acid; NM = nonmolted; TT = tetrathionate; VFA = volatile fatty acid; ZAC = zinc acetate; ZPR = zinc propionate.

(Holt, 1993; Thiagarajan et al., 1994; Holt et al., 1995; Durant et al., 1999), and increase horizontal transfer in flocks (Holt et al., 1998).

The poultry industry needs assessment of alternative molting procedures that do not require feed withdrawal but allow layer house managers to retain the economic advantages of obtaining a second laying cycle with a high production rate of high quality eggs via molting without increasing the risk of SE (Keshavarz and Quimby, 2002).

Several dietary alterations have been used to induce molt including feeding plant byproducts, alteration of the dietary mineral balance, or feeding salt- or sodium-deficient diets (Rolon et al., 1993). Including zinc at 1 to 2% (10,000 to 20,000 ppm) of the ration has often been examined as a possible alternative to feed deprivation (Shippee et al., 1979; Stevenson and Jackson, 1984; Berry and Brake, 1985; McCormick and Cunningham, 1987).

Zinc oxide, when fed at 20,000 ppm, completely stops egg production within 5 d (Scott and Creger, 1976; Creger and Scott, 1977; Berry and Brake, 1985), and hens fed high zinc stop ovulating up to a full day sooner than fasted hens (Berry and Brake, 1985; Creger and Scott, 1977). Similar results have been obtained using zinc acetate or zinc oxide (10,000 ppm zinc) (Shippee et al., 1979), zinc sulfate heptahydrate (≤2,800 ppm zinc) in a low calcium diet (Breeding et al., 1992), or zinc propionate (10,000 ppm zinc; Park et al., 2004).

Alternative molting diets have been developed from wheat middlings (Seo et al., 2001) or alfalfa (Kwon et al., 2001; Medvedev et al., 2001) and have been shown to limit SE colonization. This supports the hypothesis that the key to inducing a molt that minimizes SE infection of laying hens is to use a molt diet that not only retains a crop microflora near the population levels found in full-fed birds but maximizes crop bacterial fermentation activities antagonistic to SE colonization (Ricke, 2003). Molt diets that retain protective microflora during induced molting would provide poultry producers with dietary approaches that would potentially avoid the more drastic measure of feed withdrawal that is accompanied by increases in SE contamination (Holt, 2003; Ricke, 2003). The present study was conducted to determine if alternative molting diets, utilizing zinc acetate (ZAC) or zinc propionate (ZPR) salts at high concentrations of zinc (10,000 ppm), would reduce intestinal colonization by SE in laying hens, and to determine if key characteristic changes in the chicken intestinal microenvironment can be linked to diet, feed consumption, and SE colonization.

MATERIALS AND METHODS

Bacterial Strain

A primary poultry isolate of SE (phage type 19A), obtained from the National Veterinary Services Laboratory, Ames, IA, and selected for resistance to novobiocin and nalidixic acid (NONA) in the USDA-ARS, College Station, TX, was used. Media used to culture the resistant isolate in experimental studies contained 25 μg of NO and 20 μg of NA/mL. The challenge inocula were prepared from an overnight culture that had been previously transferred 3 times in trypticase soy broth. The culture was serially diluted in sterile phosphate-buffered saline to a concentration of approximately 10^4 cfu/mL. The number of cfu in the challenge inoculum was confirmed by plating onto brilliant green agar (BGA) plates.[5]

Molt Procedure

Feed deprivation by a modification (Holt, 1993) of a previously described procedure (Brake et al., 1982) was used to induce molt. Seven days before feed removal or feeding the high zinc diet, hens were exposed to an 8L:16D photoperiod, which was continued throughout the experiment. Beginning on d 0, feed was withdrawn for 9 d, or hens received their respective molting diets, after until trial termination.

Experimental Protocol

Single Comb White Leghorn hens (Hy-Line International, strain W-36) over 50 wk of age were obtained from a commercial laying flock. Cloacal swab samples were collected from each hen and examined for salmonellae by successive culturing in tetrathionate (TT) broth[5] and BGA as described by Andrews et al. (1992). *Salmonella* spp.-positive hens were eliminated from the study. Laying hens were placed in wire layer cages (2 hens per cage) and provided free access to water and a balanced, unmedicated, corn-soybean mash layer feed ration that met or exceeded NRC requirements (1994). This diet was formulated to provide 2,818 kcal of ME/kg, 16.5% CP, 3.5% calcium, and 0.48% available phosphorus. Before use, 3 randomly selected 25-g samples of the feed were cultured successively in buffered peptone water, tetrathionate broth, and BGA as described by Andrews et al. (1992) and examined for salmonellae. *Salmonella* spp.-positive feed was not found. The hens were allowed to acclimate for a minimum of 1 wk, followed by complete random allocation to 4 treatment groups of 12 hens each, designated as follows: (1) nonmolted control (NM); (2) nonfed (molted, M); (3) zinc acetate diet (ZAC); or (4) zinc propionate diet (ZPR), in 3 separate trials. The zinc diets contained 10,000 mg of zinc per kg of diet (10,000 ppm). The hens were housed in approved facilities at the USDA-ARS, College Station, Texas, under a protocol approved by the USDA-ARS Animal Use and Care Committee.

On d 4 of each study, all hens in each treatment group were challenged by crop gavage with 1 mL of inoculum containing approximately 10^4 cfu of NONA-resistant SE. The challenge dosage approximates the 5.6×10^4 cfu dose reported previously to be the mean infectious dosage (ID50) for SE in nonmolted hens (Holt, 1993). On the last day of each study, all hens challenged with SE in each

[5]Difco Laboratories, Detroit, MI.

group were euthanized and the crop, ceca, liver, spleen, and ovary aseptically excised. The crop, ceca, liver, spleen, and ovary of each hen were cultured for SE.

Crop Lactic Acid Concentrations and pH

Crop lactic acid concentrations and pH were determined as described previously (Durant et al., 1999). Crop pH was determined by insertion of a sterile glass pH electrode[6] through an incision in the crop wall ensuring that the electrode remained in contact with the crop mucosal surface (Durant et al., 1999). Each crop was aseptically excised, cut open, and the entire crop and contents together with 10 mL of sterile distilled water were blended for 1 min in a Stomacher 80 laboratory blender.[7] Samples of blended crop were analyzed for lactic acid concentrations.

Cecal Volatile Fatty Acid and Lactic Acid Concentrations

The concentrations of volatile fatty acids (VFA; acetic, propionic, butyric, isobutyric, valeric, and isovaleric acids) in the cecal contents were determined by gas-liquid chromatography as described previously (Corrier et al., 1990). Briefly, the analyses were conducted with a gas chromatograph equipped with a flame ionization detector and peak profiles integration-quantification integrator.[8] Each sample peak profile was integrated and quantified relative to an internal standard of methylbutyric acid placed in the same sample. Analyses were conducted at an oven temperature of 200°C and a flow rate of 85 mL/min. The concentration of each acid was expressed as micromoles per milliliter. Lactic acid concentrations were determined by an enzymatic method (Hohorst, 1974).

Crop Colonization by SE

One milliliter of the blended crop sample was transferred into 10 mL of TT broth and incubated for 24 h at 37°C. After incubation, the broth was streaked onto NONA-BGA plates, incubated for an additional 24 h at 37°C, and examined for the presence of suspect SE colonies. Suspect colonies were confirmed by biochemical tests on triple sugar-iron agar and lysine-iron agar[9] and further identified as SE serologically using *Salmonella* O antiserum group D, factors 1, 9, 12. Identification of the NONA-resistant SE by the culture on NONA-BGA plates and by the biochemical and serological procedures described were considered confirmatory without further serotyping.

Cecal Colonization by SE

One cecum from each hen was cut into several pieces, placed in 30 mL of TT broth, shaken vigorously, and incubated for 24 h at 37°C. After incubation, the broth was streaked on NONA-BGA plates, incubated for an additional 24 h at 37°C, and examined for the presence of suspect SE colonies. Suspect colonies were confirmed biochemically and serologically as described in the section on crop colonization.

Liver, Spleen, and Ovary Colonization by SE

Liver, spleen, and ovary specimens were minced with scissors and cultured. The organ samples were incubated for 24 h at 37°C in TT broth. After incubation, the broth was streaked onto NONA-BGA plates, incubated for an additional 24 h at 37°C, and examined for the presence of SE colonies. Suspect colonies were confirmed biochemically and serologically as described in the section on crop colonization.

SE Colony-Forming Units Per Gram of Crop and Cecal Contents

The contents of the crop and one cecum from each hen were serially diluted and spread plated on NONA-BGA plates at dilutions 10^{-1} through 10^{-4}. The plates were incubated for 24 h at 37°C, after which the number of colony forming units of SE per gram of crop or cecal content was determined and SE colonies were confirmed biochemically and serologically as described in the section on crop colonization. Crop and cecal content samples in which SE was not detected at the 10^{-1} dilution on BGA plates and after TT broth enrichment and BGA plating were scored as 0 cfu. Crop and cecal content samples negative at 10^{-1} dilution on BGA plates but positive after TT enrichment and BGA plating were arbitrarily assigned log 0.95 cfu of SE per gram of crop or cecal contents.

Statistical Analysis

Chi-square analysis was used to determine significant differences between treatment groups for incidences of SE colonization of the crop, ceca, liver, spleen, and ovary (Luginbuke and Schlotzhauer, 1987). Differences in the crop pH, VFA concentrations, and $\log_{10}$ cfu of SE counts among treatment groups were determined by ANOVA using the GLM procedure. Significant differences were further separated using Duncan's multiple range test and commercial statistical analysis software[10] (Luginbuke and Schlotzhauer, 1987). All data analyzed by statistical analyses were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Feed Intake

Natural molting is caused by self-induced decreased feed intake and activity during the late summer and early

[6]Model 05669-20; Cole Palmer, Niles, IL.
[7]Stomacher 80 Lab Blender, Stewart Medical, London, UK.
[8]Model 110 Gas Chromatograph, SR1 Instruments, Torrance, CA.
[9]Oxoid, Unipath Ltd., Hampshire, UK.
[10]SAS Institute Inc., Cary, NC.

TABLE 1. Effects of nonmolting and molting diets on feed intake, body weight loss, and ovary weight of hens

	Treatment[1]			
Parameter	NM	M	ZAC	ZPR
Trial 1				
Feed intake (g/hen per day)	67.35 ± 16.05[a]	NA[2]	42.20 ± 0.42[ab]	13.85 ± 9.12b[b]
Body weight loss (%)	0.21 ± 6.06[a]	25.10 ± 4.33[c]	10.82 ± 8.66[b]	16.36 ± 9.09[b]
Ovarian weight (g)	29.94 ± 24.68[a]	7.35 ± 5.40[b]	10.30 ± 5.05[b]	9.18 ± 5.08[b]
Trial 2				
Feed intake (g/hen per day)	81.72 ± 9.40[a]	NA	34.40 ± 1.70[b]	21.40 ± 1.85[b]
Body weight loss (%)	1.53 ± 7.01[a]	21.63 ± 4.70[c]	10.99 ± 7.18[b]	14.62 ± 6.16[b]
Ovarian weight (g)	38.07 ± 16.97[a]	7.79 ± 3.17[a]	12.10 ± 8.11[b]	9.69 ± 6.80[b]
Trial 3				
Feed intake (g/hen per day)	88.40 ± 8.20[a]	NA	57.20 ± 6.08[b]	14.60 ± 1.13[c]
Body weight loss (%)	4.11 ± 3.61[a]	30.34 ± 3.67[d]	12.09 ± 6.60[b]	23.18 ± 5.27[c]
Ovarian weight (g)	40.26 ± 5.86[a]	7.04 ± 3.08[c]	15.57 ± 12.69[b]	8.46 ± 5.15[c]

[a-c]Means within a row with no common superscripts differ significantly ($P < 0.05$).

[1]NM = nonmolted, hens received Texas A&M University (TAMU) layer ration for 9 d; M = molted, hens underwent feed withdrawal for 9 d; ZAC = hens received a diet containing 10,000 mg/kg zinc as zinc acetate for 9 d; ZPR = hens received a diet containing 10,000 mg/kg zinc as zinc propionate for 9 d.

[2]NA = not applicable.

fall when day length naturally begins to shorten (Brake and Thaxton, 1979; Mrosovsky and Sherry, 1980). These physiological changes cause the cessation of reproduction (Brake and Thaxton, 1979; Mrosovsky and Sherry, 1980). Therefore, wild birds take a self-induced rest to rejuvenate body tissues and build up energy stores. Feed deprivation appears to be important in ensuring an adequate molt.

Full-fed (nonmolted; NM) hens had ($P < 0.05$) greater feed intake (67.35, 81.72, and 88.40 g/hen per day in trials 1, 2, and 3, respectively) than did ZAC- or ZPR-fed hens in all 3 trials (Table 1). However, feed intake of trial 1 NM birds was somewhat lower than for NM birds in trials 2 and 3. This may in part be due to the higher individual feed intake variation observed in trial 1 NM birds compared with the NM birds in the other 2 trials. The ZPR-fed hens (14.60 g/hen per d) had ($P < 0.05$) less feed intake than did ZAC-fed hens (57.20 g/hen per d) in trial 3. Compared with the feed intake of NM hens, there was reduced feed intake in ZPR-fed hens (trial 1: 79.44, trial 2: 73.81, trial 3: 83.48%) and ZAC-fed hens (trial 1: 37.34, trial 2: 57.66, trial 3: 35.29%) in all 3 trials. The reduced feed intake could be due to appetite depression (Brink et al., 1950), or low palatability of high levels of propionic acid (Ryś and Koreleski, 1974; Cave, 1982, 1984) or zinc (Fox, 1989). It has also been reported that the reduced feed intake could be due to the ability of zinc cation (Zn^{2+}) to induce follicular atresia and halt egg laying (Scott and Creger, 1976; Shippee et al., 1979; Berry and Brake, 1985; McCormick and Cunningham, 1987; Johnson and Brake 1992).

BW Loss

Body weight loss influences the outcome of an induced molting procedure (Brake and Thaxton, 1979; Brake et al., 1981; Brake and McDaniel, 1981; Baker et al., 1983). Approximately 25% BW loss in hens by the feed withdrawal has been directly attributed to decreased muscle and liver weight, decreased use of adipose tissue, involution of the reproductive organs, and greater reproductive regression (Brake and Thaxton, 1979; Berry and Brake, 1985).

Nonfed (M) hens exhibited ($P < 0.05$) more BW loss (trial 1: 25.10, trial 2: 21.63, trial 3: 30.34%) than did ZAC- or ZPR-fed hens in all 3 trials (Table 1). However, there was no ($P > 0.05$) difference in BW loss between ZAC-fed hens (trial 1: 10.82, trial 2: 10.99%) and ZPR-fed hens (trial 1: 16.36, trial 2: 14.62%) in trials 1 and 2. In trial 3, there were ($P < 0.05$) differences in BW loss for all 4 treatments. NM hens had the least BW loss compared with other treatments in all 3 trials. Similar responses in hens' BW loss were observed by Park et al. (2004) for hens undergoing feed withdrawal (25.12%), 1% zinc as Zn acetate (15.52%) or Zn propionate hens (15.66%), and nonmolted control hens (1.15%). The extent of a hens' BW loss by ZAC or ZPR feeding was similar to values in a previous study by McCormick and Cunningham (1987), who reported that BW loss during the 4 d for fasted and zinc-fed hens was 16.4 and 15.2%, respectively.

Ovarian Weight

The degree of ovarian regression has been associated with overall reproductive rejuvenation during an induced molt (Brake and Thaxton, 1979). McCormick and Cunningham (1984) reported that feeding of 1% zinc or 2% zinc as Zn oxide for 4 d resulted in 80% reduction in ovarian weights of hens. Therefore, ovarian weight was measured in the current study as a comparison of the degree of molting for each treatment. ZAC- (trial 1: 10.30, trial 2: 12.10 g) or ZPR- (trial 1: 9.18, trial 2: 9.69 g) fed hens did not have ($P > 0.05$) different ovarian weights when compared with M hens (trial 1: 7.35, trial 2: 7.79 g) in trials 1 and 2. All other molted dietary treatments had

TABLE 2. Effects of nonmolting and molting diets on the crop lactic acid concentration and pH of hens

	Treatment[1]			
Parameter	NM	M	ZAC	ZPR
Trial 1				
Lactic acid (μmol/mL)	29.53 ± 37.33	11.05 ± 3.00	19.23 ± 11.0	16.98 ± 4.81
Crop pH	5.19 ± 0.45	5.42 ± 0.72	5.82 ± 1.15	5.90 ± 0.54
Trial 2				
Lactic acid (μmol/mL)	22.02 ± 12.96^{a}	6.67 ± 1.49^{c}	15.54 ± 5.14^{b}	9.55 ± 5.18^{bc}
Crop pH	4.79 ± 0.48^{b}	5.49 ± 0.72^{a}	5.36 ± 0.73^{a}	5.40 ± 0.54^{a}
Trial 3				
Lactic acid (μmol/mL)	20.70 ± 10.82^{a}	7.97 ± 1.65^{b}	13.96 ± 9.0^{ab}	17.81 ± 13.31^{a}
Crop pH	4.29 ± 0.52^{bc}	4.81 ± 0.42^{ab}	4.06 ± 0.98^{c}	5.17 ± 0.61^{a}

$^{a\text{-}c}$Means within a row with no common superscripts differ significantly ($P < 0.05$).

[1]NM = nonmolted, hens received Texas A&M University (TAMU) layer ration for 9 d; M = molted, hens underwent feed withdrawal for 9 d; ZAC = hens received a diet containing 10,000 mg/kg zinc as zinc acetate for 9 d; ZPR = hens received a diet containing 10,000 mg/kg zinc as zinc propionate for 9 d.

($P < 0.05$) lower ovarian weights when compared with NM (trial 1: 29.94, trial 2: 38. 07 g) hens in trials 1 and 2 (Table 1). Similar ovarian weight responses to those in trial 1 were seen by Park et al. (2004) where nonmolted control hens had significantly higher ovarian weights (31.04 g) compared with all other molted hen dietary treatments (Zn acetate, Zn propionate, and feed withdrawal).

Crop Lactic Acid and pH Level

Lactic acid is the main fermentation product of the *Lactobacillus* spp. that are present in the crop (Fuller, 1977). Lactic acid concentrations were ($P < 0.05$) lower in the crop of M hens (trial 2: 6.67, trial 3: 7.97 μmol/mL) than in that of NM hens (trial 2: 22.02, trial 3: 20.70 μmol/mL) in trials 2 and 3 (Table 2), ZAC hens (15.54 μmol/mL) in trial 2, and ZPR hens (17.81 μmol/mL) in trial 3. The significant decrease in lactic acid in the M hens may have resulted from a significant decrease in the numbers of *Lactobacilli* in these birds although the numbers of lactobacilli were not determined in the current study.

There were no ($P > 0.05$) differences in the crop pH in all 4 treatments in trial 1 (Table 2). However, in trial 2, NM hens (4.79) had lower crop pH values ($P < 0.05$) than the molted hens. In trial 3, the ZAC hens (4.06) had ($P < 0.05$) lower values of crop pH than the ZPR (5.17) and M hens (4.81). Reduced feed intake is thought to be the main factor causing decreases in crop *Lactobacilli* populations, thereby allowing crop pH to rise in hens that are deprived of feed (Humphrey et al., 1993; Corrier et al., 1999; Durant et al., 1999). Humphrey et al. (1993) reported that when chickens are undergoing malnutrition or starvation, the crop pH can increase due to decreased *Lactobacillus* fermentation within the crop. Feed withdrawal for 9 d resulted in a decrease in lactic acid in the crop, accompanied by an increase in crop pH (Durant et al., 1999). Feeding ZAC or ZPR may be inhibitory to *Lactobacilli* population due to the influence of zinc on microorganism growth and infectivity in animals. *Lactobacilli* that are the predominant microflora in crop and play an important role in maintaining the low pH that prevents coliform establishment in the crop and *E. coli* growth in vitro (Fuller and Brooker, 1974; Fuller, 1977). Decreases in the concentrations of lactic acid during feed withdrawal were not always accompanied by a significant increase in the crop pH in the M hens in the current study. Therefore, increases in crop pH by dietary molting treatments such as M, ZAC, or ZPR feeding may simply decrease lactic acid production by crop microflora without altering the population levels substantially. Hume et al. (2003) (using denaturing gradient gel electrophoresis) noted that in hens where addition of zinc (2800 mg/kg) was used to induce molting a typical microflora was still present despite the decrease in hen's feed intake caused by excess zinc levels in the feed. They also reported that even nonmolted and feed withdrawal molted hens exhibited 40% similarity between cecal communities, whereas diets with low calcium (0.8% wt/wt) and high zinc (2,800 mg/kg) exhibited 90% similarity between cecal communities.

Cecal VFA and Lactic Acid

There were no ($P > 0.05$) differences in concentrations of acetic acid in the ceca of hens from all 4 treatments in 3 trials (Table 3). Concentrations of propionic acid were ($P < 0.05$) lower in the ceca of M hens (trial 2: 21.12, trial 3: 25.46 μmol/mL) than in ceca of NM hens (trial 2: 45.38, trial 3:50.81 μmol/mL), ZAC- (45.08, 52.13 μmol/mL), or ZPR-fed hens (43.65, 51.77 μmol/mL), but the concentrations of propionic acid were not ($P > 0.05$) different in the ceca of hens from all 4 treatments in trial 1 (Table 3). Concentrations of butyric acid were ($P < 0.05$) lower in the ceca of M hens (13.11 μmol/mL) than in that of ZAC (18.86 μmol/mL) or NM (19.81 μmol/mL) hens in trial 3, but concentrations of butyric acid were not ($P > 0.05$) different in the ceca of hens from all 4 treatments in trials 1 and 2 (Table 3). Concentrations of isobutyric acid were ($P < 0.05$) higher in the ceca of ZPR-fed hens (8.23 μmol/mL) than in that of M hens (5.71 μmol/mL) in trial 3, but concentrations of isobutyric acid were not ($P > 0.05$) different in the ceca of hens from all 4 treatments in trials

TABLE 3. Effects of nonmolting and molting diets on cecal volatile fatty acid (VFA) and lactic acid concentrations (μmol/mL)

	Treatment[1]			
Item	NM	M	ZAC	ZPR
Trial 1				
Acetic acid	69.05 ± 25.02	50.50 ± 37.52	77.73 ± 30.38	76.23 ± 39.74
Propionic acid	21.13 ± 10.20	11.93 ± 17.82	22.29 ± 13.87	23.45 ± 17.35
Butyric acid	6.33 ± 4.88	5.01 ± 8.05	6.81 ± 5.25	8.27 ± 7.17
Isobutyric acid	2.83 ± 1.10	1.99 ± 2.26	2.75 ± 1.77	3.07 ± 2.52
Valeric acid	2.68 ± 1.24	1.83 ± 2.52	2.94 ± 1.91	3.10 ± 2.06
Isovaleric acid	2.56 ± 1.10	1.68 ± 1.80	2.23 ± 1.67	2.72 ± 2.10
Total VFA	106.56 ± 42.75	74.40 ± 69.52	117.02 ± 54.47	119.64 ± 71.01
Lactic acid	6.08 ± 1.62	4.07 ± 1.47	4.32 ± 1.28	5.23 ± 3.28
Trial 2				
Acetic acid	103.70 ± 27.30	91.39 ± 48.61	112.63 ± 30.82	112.79 ± 55.45
Propionic acid	45.38 ± 16.23[a]	21.12 ± 14.19[b]	45.08 ± 16.86[a]	43.65 ± 24.65[a]
Butyric acid	21.26 ± 6.93	18.08 ± 9.68	20.52 ± 8.41	15.59 ± 9.57
Isobutyric acid	5.49 ± 6.30	3.80 ± 2.49	4.69 ± 2.88	6.25 ± 7.03
Valeric acid	4.88 ± 1.96	3.48 ± 2.24	5.54 ± 2.62	5.28 ± 2.72
Isovaleric acid	2.80 ± 1.26	2.78 ± 2.00	3.12 ± 1.38	2.83 ± 1.79
Total VFA	184.13 ± 53.61	140.34 ± 75.35	194.12 ± 63.31	190.48 ± 95.74
Lactic acid	12.03 ± 4.17[a]	7.19 ± 3.25[b]	6.67 ± 1.83[b]	8.26 ± 6.35[b]
Trial 3				
Acetic acid	98.56 ± 27.03	61.80 ± 24.89	106.71 ± 36.05	210.72 ± 355.9
Propionic acid	50.81 ± 12.76[a]	25.46 ± 13.20[b]	52.13 ± 18.15[a]	51.77 ± 19.76[a]
Butyric acid	19.81 ± 3.46[a]	13.11 ± 5.64[b]	18.86 ± 6.68[a]	17.19 ± 4.96[b]
Isobutyric acid	7.04 ± 1.40[ab]	5.71 ± 1.66[b]	6.74 ± 2.71[ab]	8.23 ± 4.00[a]
Valeric acid	7.62 ± 1.48[a]	5.37 ± 2.26[b]	7.68 ± 2.00[a]	8.16 ± 2.86[a]
Isovaleric acid	6.34 ± 1.37[ab]	5.08 ± 1.57[b]	6.19 ± 1.44[ab]	7.04 ± 1.90[a]
Total VFA	192.59 ± 44.4[a]	118.30 ± 48.8[b]	201.45 ± 63.6[a]	207.03 ± 69.2[a]
Lactic acid	16.09 ± 11.72[a]	4.59 ± 4.39[b]	9.90 ± 6.07[ab]	6.87 ± 6.08[b]

[a,b]Means within a row with no common superscripts differ significantly ($P < 0.05$).

[1]NM = nonmolted, hens received Texas A&M University (TAMU) layer ration for 9 d; M = molted, hens underwent feed withdrawal for 9 d; ZAC = hens received a diet containing 10,000 mg/kg zinc as zinc acetate for 9 d; ZPR = hens received a diet containing 10,000 mg/kg zinc as zinc propionate for 9 d.

1 and 2 (Table 3). Concentrations of valeric acid were ($P < 0.05$) lower in the ceca of M hens (5.37 μmol/mL) than in that of NM hens (7.62 μmol/mL), ZAC-fed hens (7.68 μmol/mL), or ZPR-fed hens (8.16 μmol/mL) in trial 3, but concentrations of valeric acid were not ($P > 0.05$) different in the ceca of hens from all 4 treatments in trials 1 and 2 (Table 3). Concentrations of isovaleric acid were ($P < 0.05$) lower in the ceca of M hens (5.08 μmol/mL) than in that of ZPR-fed hens (7.04 μmol/mL) in trial 3, but concentrations of isovaleric acid were not ($P > 0.05$) different in the ceca of hens from all 4 treatments in trials 1 and 2 (Table 3). Concentrations of total VFA were ($P < 0.05$) lower in the ceca of M hens (118.30 μmol/mL) than in that of NM hens (192.59 μmol/mL), ZAC hens (201.45 μmol/mL), or ZPR-fed hens (207.03 μmol/mL) in trial 3, but concentrations of total VFA were not ($P > 0.05$) different in the ceca of hens from all 4 treatments in trials 1 and 2 (Table 3). Concentrations of lactic acid were ($P > 0.05$) higher in the ceca of NM hens (trial 2:12.03, trial 3:16.09 μmol/mL) than M hens (trial 2:7.19, trial 3:4.59 μmol/mL), ZAC-fed hens (trial 2: 6.67 μmol/mL), and ZPR-fed hens (trial 2:8.26 μmol/mL), but concentrations of lactic acid were not ($P > 0.05$) different in the ceca of hens from all 4 treatments in trial 1 (Table 3). Corrier et al. (1997) reported that the concentrations of total VFA along with acetic, propionic, and butyric acids were significantly decreased in the ceca of feed withdrawal molted hens compared with unmolted hens, but there was no significant differences in cecal pH or cecal oxidation-reduction potential between molted and unmolted hens.

SE Colonization in the Crop

Dietary zinc may influence growth and infectivity of bacterial pathogens in animal. Park et al. (2002) recently reported that zinc may inhibit *S. typhimurium* under in vitro aerobic or anaerobic atmospheric conditions. Earlier studies have shown that molting diets containing high zinc acetate (10,000 ppm) (Kubena et al., 2001) or lower concentrations of zinc acetate (110 ppm) (Ricke et al., 2001) decreased SE colonization in laying hens compared with hens undergoing feed withdrawal. Compared with NM hens (0%), the number of SE-positive crop cultures increased ($P < 0.05$) in M hens (91.7%), ZAC hens (33.3%), or ZPR hens (100%) in trial 1 (Table 4). The ZPR hens had higher ($P < 0.05$) SE populations (2.31 cfu/g) in the crop contents than did NM hens (0 cfu/g), M hens (0.52 cfu/g), or ZAC hens (0 cfu/g) in trial 1 (Table 4). When compared with NM hens (16.7%), the number of SE-positive crop cultures increased ($P < 0.05$) in the M hens (83.3%), or ZPR hens (100%), which had a higher ($P < 0.05$) SE population (1.51 cfu/g) in the crop contents than did the NM hens (0 cfu/g) or ZAC hens (0 cfu/g) in trial 2 (Table 4). There were no ($P > 0.05$) differences in SE

TABLE 4. Effects of nonmolting and molting diets on *Salmonella enteritidis* crop colonization of hens[1]

	Treatment[2]			
Parameter	NM	M	ZAC	ZPR
Trial 1				
Positive hens/total (%)	0/12 (0)	11/12 (91.7)*	4/12 (33.3)*	12/12 (100)*
Log_{10} cfu/g	0^{b}	0.52 ± 1.31^{b}	0^{b}	2.31 ± 2.95^{a}
Trial 2				
Positive hens/total (%)	2/12 (16.7)	10/12 (83.3)*	1/12 (8.3)	12/12 (100)*
Log_{10} cfu/g	0^{b}	0.63 ± 1.03^{ab}	0^{b}	1.51 ± 2.27^{a}
Trial 3				
Positive hens/total (%)	0/11 (0)	1/12 (8.3)	0/11 (0)	0/12 (0)
Log_{10} cfu/g	0	0.28 ± 0.98	0	0

[1]Hens were challenged by crop gavage with 10^4 cfu of *S. enteritidis* on d 4 of molt and cultured for *Salmonella* on d 9 of molt.

[2]NM = nonmolted, hens received Texas A&M University (TAMU) layer ration for 9 d; M = molted, hens underwent feed withdrawal for 9 d; ZAC = hens received a diet containing 10,000 mg/kg zinc as zinc acetate for 9 d; ZPR = hens received a diet containing 10,000 mg/kg zinc as zinc propionate for 9 d.

*$P < 0.05$, and **$P < 0.01$, between treatment and control (NM) (n = 12 or n = 11).

colonization and number (cfu/g) in the crop contents from 4 treatments in trial 3 (Table 4).

The crop can be one of the main reservoirs for *Salmonella* (Hargis et al., 1995), and feed withdrawal can increase in the number of chickens with crops colonized by *Salmonella* (Ramirez et al., 1997). Humphrey et al. (1993) reported that an increase in the recovery of SE from the crop of broilers resulted from feed deprivation for 24 h. Durant et al. (1999) reported that the introduction of SE into the crop environment with high pH and lowered concentrations of lactate were accompanied by increased crop colonization.

SE Colonization in the Ceca

When compared with NM hens (8.3%), the number of hens with SE-positive cecal cultures increased ($P < 0.05$) in the M (100%), ZAC (25%), and ZPR groups (100%) but there were no ($P > 0.05$) differences in *S. enteritidis* number (cfu/g) in the cecal contents from 4 treatments in trial 1 (Table 5). Likewise, when compared with NM (41.7%) and ZAC hens (33.3%), the number of SE-positive cecal cultures increased ($P < 0.05$) in the M hens (91.7%), and ZPR hens (58.3%) but there were no ($P > 0.05$) differences in SE number (cfu/g) in the cecal contents from 4 treatments in trial 2. In trial 3, compared with NM hens (18.2%), the number of SE-positive cecal cultures increased ($P < 0.05$) in the M hens (83.3%), which had a significantly higher SE number (cfu/g) in the cecal contents than the NM birds but not the ZAC and ZPR birds.

Besides the crop, the ceca is the alimentary tract site in poultry that is most likely to be colonized by *Salmonella* (Fanelli et al., 1971), and SE replicates and disseminates to various organs, including the ovaries (Gast and Beard, 1990; Shivaprasad et al., 1990). Feed withdrawal may cause an increase in the level of cecal colonization of *Salmonella* (Moran and Bilgili, 1990; Ramirez et al., 1997). The native intestinal bacteria have a protective function against *Salmonella* colonization of the ceca (Nurmi and Rantala, 1973; Barnes et al., 1980; Impey and Mead, 1989; Nisbet et al., 1994; Corrier et al., 1995) in chickens. Prevention of *Salmonella* colonization of the cecum has been

TABLE 5. Effects of molting and nonmolting diets on *Salmonella enteritidis* cecal colonization of hens[1]

	Treatment[2]			
Item	NM	M	ZAC	ZPR
Trial 1				
Positive hens/total (%)	1/12 (8.3)	12/12 (100)*	3/12 (25)*	12/12 (100)*
Log_{10} cfu/g	0.53 ± 1.27	1.14 ± 2.07	0.56 ± 1.35	0.67 ± 1.61
Trial 2				
Positive hens/total (%)	5/12 (41.7)	11/12 (91.7)*	4/12 (33.3)	7/12 (58.3)**
Log_{10} cfu/g	0.37 ± 1.27	1.89 ± 2.96	0.89 ± 2.14	2.26 ± 2.51
Trial 3				
Positive hens/total (%)	2/11 (18.2)	10/12 (83.3)*	4/11 (36.4)	6/12 (50)
Log_{10} cfu/g	0^{b}	2.77 ± 3.44^{a}	0.55 ± 1.27^{ab}	1.82 ± 2.99^{ab}

[1]Hens were challenged by crop gavage with 10^4 cfu of *S. enteritidis* on d 4 of molt and cultured for *Salmonella* on d 9 of molt.

[2]NM = nonmolted, hens received Texas A&M University (TAMU) layer ration for 9 d; M = molted, hens underwent feed withdrawal for 9 d; ZAC = hens received a diet containing 10,000 mg/kg zinc as zinc acetate for 9 d; ZPR = hens received a diet containing 10,000 mg/kg zinc as zinc propionate for 9 d.

* $P < 0.05$, and **$P < 0.01$, between treatment and control (NM) (n = 12 or n = 11).

TABLE 6. Effects of nonmolting and molting diets on *Salmonella enteritidis* colonization of the liver, spleen, and ovary of hens[1]

	Treatment[2]			
Item	NM	M	ZAC	ZPR
Trial 1				
Liver	0/12 (0)	8/12 (66.7)*	1/12 (8.3)	11/12 (91.7)*
Spleen	0/12 (0)	7/12 (58.3)*	1/12 (8.3)	11/12 (91.7)*
Ovary	2/12 (16.7)	10/12 (83.3)*	2/12 (16.7)	12/12 (100)*
Trial 2				
Liver	3/12 (25)	9/12 (75)*	1/12 (8.3)	9/12 (75)*
Spleen	1/12 (8.3)	6/12 (50)*	5/12 (41.7)**	8/12 (66.7)*
Ovary	12/12 (100)	7/12 (58.3)*	8/12 (66.7)*	9/12 (75)**
Trial 3				
Liver	1/11 (9.1)	6/12 (50)*	3/11 (27.8)	3/12 (25)
Spleen	0/11 (0)	2/12 (16.7)	2/12 (16.7)	3/12 (25)**
Ovary	0/11 (0)	6/12 (50)*	2/12 (16.7)	1/12 (8.3)

[1]Hens were challenged by crop gavage with 10^4 cfu of *S. enteritidis* on d 4 of molt and cultured for *Salmonella* on d 9 of molt.

[2]NM = nonmolted, hens received Texas A&M University (TAMU) layer ration for 9 d; M = molted, hens underwent feed withdrawal for 9 d; ZAC = hens received a diet containing 10,000 mg/kg zinc as zinc acetate for 9 d; ZPR = hens received a diet containing 10,000 mg/kg zinc as zinc propionate for 9 d.

*$P < 0.05$, and **$P < 0.01$, between treatment and control (NM) (n = 12 or n = 11).

positively correlated with increased VFA concentrations and decreased pH (Barnes et al., 1979; Nisbet et al., 1994; Corrier et al., 1995). Lowered pH can promote the bacteriostatic action of VFA by increasing the concentrations of the undissociated state, which can permeate the cell membrane (Cherrington et al., 1991). The decreased number of *Salmonella* in the cecal contents of chicks has been associated with elevated concentrations of propionic acid and total VFA (Hollister et al., 1992; Ziprin et al., 1993; Nisbet et al., 1994; Corrier et al., 1995). Volatile fatty acids in the cecal contents in chicken are fermentation products of indigenous anaerobic bacteria (Barnes et al., 1979, 1980) and the concentrations of VFA may represent the degree of fermentation activity of bacteria in the ceca (Barnes et al., 1980). Corrier et al. (1997) reported that induced molting by feed withdrawal had no apparent effect on pH or on the oxidation-reduction potential of the ceca. The lactic acid bacteria probably do not play a major role in the metabolic activity in the ceca because the concentration of fermentable carbohydrates that favors their growth is much lower than in the crop. Cecal contents of broilers normally contain very low concentrations of lactic acid (Hinton et al., 1991), which is the major metabolic byproduct of these acid-producing bacteria.

SE Colonization in Liver, Spleen, and Ovary

Compared with NM hens (liver: 0%, spleen: 0%, ovary: 16.7%), the number of SE-positive cultures in liver, spleen, and ovaries increased ($P < 0.05$) in the M hens (liver: 66.7%, spleen: 58.3%, ovary: 83.3%), and ZPR hens (liver: 91.71%, spleen: 91.7%, ovary: 100%) in trial 1 (Table 6). Compared with NM hens (liver: 25%, spleen: 8.3%), the number of SE-positive cultures in liver and spleen increased ($P < 0.05$/ $P < 0.01$) in the M hens (liver: 75%, spleen: 50%), ZAC hens (spleen: 41.7%), and ZPR hens (liver: 75%, spleen: 66.7%), whereas SE-positive ovary cultures decreased in all molted hens (M, 58.3%, ZAC, 66.7%, ZPR, 75%) compared with NM (100%) in trial 2 (Table 6). Compared with NM hens (liver: 9.1%, spleen: 0%, ovary: 0%), the number of SE-positive organ cultures increased only ($P < 0.05$/ $P < 0.01$) in the M hens (liver: 50%, ovary: 50%) and ZPR hens (spleen: 25%) in trial 3 (Table 6).

The presence of feed in the gastrointestinal tract stimulates peristalsis and mucin production (Sturkie, 1965). Feed deprivation may result in decreased peristalsis, decreased mucin production (Duke and Evanson, 1976), and increased opportunity for bacterial colonization (Holt and Porter, 1992; Holt, 1993; Holt et al., 1994, 1995). Holt et al. (1994) reported that hens infected with SE and molted by feeding a ration low in calcium and energy had less severe cecal and colonic inflammation than hens molted by complete feed deprivation. Feed deprivation may also increase the severity of SE infection by altering normal intestinal bacteria populations and pH. Corrier et al. (1990) demonstrated that lowered cecal pH, induced by administration of VFA-producing cecal flora, protected against *Salmonella typhimurium* infection in chicks. Macri et al. (1997) reported that induced molting by feed deprivation shortened the time of onset and possibly increased the severity of acute SE inflammation in the cecum, colon, and possibly the ileum. Holt and Porter (1992) reported that molted infected chickens and unmolted infected chickens exhibited no significant difference in the spleen SE counts. Cecum, liver, spleen, and ovary counts were significantly higher in the feed deprived hens and no differences in any of the SE counts were observed in nonmolted vs. hens fed wheat middlings (Seo et al., 2001). Kwon et al. (2001) reported that the total number of SE-positive organs was decreased in alfalfa-fed hens as com-

pared with fasted hens, whereas there was no colonization in nonmolted hens. Corrier et al. (1997) suggested that increased susceptibility of molted hens to SE colonization may be related to decreased fermentation and production of VFA by fermentative bacteria present in the ceca, and addition of compounds such as lactose in the drinking water may enhance resistance to SE colonization.

Some consistent trends over the 3 trials were exhibited in the SE colonization in each organ of M, NM, ZAC, or ZPR hens. Colonization of crop and ceca, in addition to spleen invasion, were more frequent in ZPR hens in the 3 trials compared with NM hens or ZAC hens whereas crop, ceca, liver, and ovary were generally more colonized in M birds vs. NM and ZAC birds. This may indicate that acetate was a more antagonistic organic acid than propionate to SE under these gastrointestinal conditions. However, zinc feeding as either ZAC or ZPR did not appear to alter the intestinal microenvironment of hens because no consistent trends were exhibited in the concentrations of crop or cecal lactic acid or individual and total VFA between NM, M, ZAC, and ZPR hens. It is also possible that feed consumption was less frequent for ZPR-fed birds than ZAC-fed birds, potentially limiting the concentration of propionate reaching the gastrointestinal tract. However, only in trial 3 was feed intake significantly reduced in ZPR birds compared with ZAC and no significant differences in cecal concentrations of either organic acid were detected in any of the trials between the 2 treatments.

The most commonly practiced method of molt induction is feed withdrawal for several days to enter into a second egg-laying cycle (North and Bell, 1990). This is an efficient method to induce a molt because it is management friendly, economically advantageous, and results in satisfactory postmolt performance for the commercial layer industry (Brake, 1993). However, increased public awareness of the animal stress associated with feed withdrawal has led researchers to investigate alternative molting processes (Holt, 2003; Ricke, 2003). Additionally, the stress results in reduced resistance of experimental hens to SE colonization (Holt and Porter, 1992; Holt, 1993; Holt et al., 1995; Corrier et al., 1997); this may indicate potential for SE problems in commercial flocks (Holt, 2003) and lead to increased risk of foodborne illness to consumers of these products. Alternative diets utilizing high concentration zinc dietary regimens have the potential to reduce the risk of SE contamination during induced molting.

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